OBSERVATIONS ON THE CARTESIAN DIVER BALANCE TECHNIQUE

by

SØREN LØVTRUP[1]

1. INTRODUCTION

During the last few years ZEUTHEN's Cartesian diver balance (9) has been used as a routine tool for measuring the relative "size" of amoebae (see HOLTER and LØVTRUP (2)). One of the results of this work is that some modifications in the diver balance technique have been introduced.

ZEUTHEN calibrated diver balances by means of gold standards weighed in a density gradient (LEVI and ZEUTHEN (3)). These gold weights had the disadvantage that they were difficult to make and handle, so that in routine work they were replaced by polystyrene standards. These are easy to handle, but difficult to clean. Dipping in concentrated sulfuric acid was proposed, but proved unsatisfactory, since, as already suggested by ZEUTHEN, it leads to loss of weight, because polystyrene is not completely stable to sulfuric acid.

To overcome this difficulty some standard weights of palladium wire have been made and weighed on a specially constructed microbalance (see section 3.21). These weights are easy to handle, and may be cleaned by heating.

A puzzling feature of the diver balance, according to ZEUTHEN (9), is the continuous change of equilibrium pressure, i.e. the divers get gradually "heavier". By introducing a correction in the formula employed by ZEUTHEN for calculation of reduced weight (RW) from observed changes in equilibrium pressure, it has been shown that the gradual change is mainly due to loss of air.

[1] Supported by a grant from the Rockefeller Foundation.

2. MODIFICATION IN BLOWING THE POLYSTYRENE CUP

By using a concentrated polystyrene solution the time required for blowing the cup of the balance may be decreased considerably. A very viscous solution (about 30 per cent polystyrene in benzene) was obtained by allowing a more dilute solution to evaporate. The pressure for blowing the cup was not applied by mouth, but by an aquarium air pump, regulated by a simple water pressure regulator (a range of 30 cm of water is sufficient). The starting pressure (~0.5 cm of water) was maintained until all the polystyrene had been drawn out of the mouth of the pipette. At this time the outer layer of polystyrene has become rigid because of benzene evaporation, and the pressure may be increased rapidly (the end value ranges from 15-30 cm)[1]. After the blowing, which must be finished in less than five minutes, the cup is dried for about one hour by means of a PHILIPS heat lamp, left for some time to cool, and cut as described by ZEUTHEN (9).

3. THE PALLADIUM WEIGHTS

3.1. Making and handling the weights.

LEVI and ZEUTHEN (3) prepared their gold weights by embedding gold foil in picein wax and cutting with a microtome. Since metal wires of proper dimensions are now commercially available, this operation can be avoided. As regards the choice of material, palladium seemed to be preferable to gold, since it is harder and therefore could be expected to show higher resistance to mechanical wear (compare LEVI and ZEUTHEN (3) who found that their gold standards might lose weight). Another advantage is that palladium has a lower density than gold. Accordingly, some palladium wire with a diameter of 10 μ was obtained from BAKER & Co, N. J. A mm length of this wire has a reduced weight of about 1 μg. Small pieces of 2–4 mm in length were cut. At one end they were bent to a hook or a loop. They were handled by means of a hook of fine platinum wire, fused into a glass capillary, and kept in porcelain dishes, the white background making it possible to see them even without magnification.

> While there are no special problems in handling the weights in air, it may be worth mentioning the technique adopted when they are used in the flotation vessels. When a weight has to be introduced into a vessel, this is filled to the rim

[1] A high content of water vapor in the air may make the blowing of the polystyrene cup impossible (ZEUTHEN (9)), LLOYD CLAFF and ZEUTHEN have successfully employed an electrical hair-drier to overcome this difficulty (personal communication by ZEUTHEN).

with flotation medium, and while the vessel is held over a porcelain dish, the weight is transferred to the medium by means of the hook. After the vessel has been put back into the thermostat, and it has been observed that the weight is lying at the bottom, the surplus medium is sucked off by means of a pipette. The weight now stays here until the vessel or the weight itself has to be cleaned. It is lifted by means of the platinum hook from the bottom to the polystyrene cup in order to be weighed.

The safest way to get the weight out of the vessel is to fill to the rim with water, cover the opening of the vessel with a finger, turn it quickly upside down, place the mouth of the vessel below the surface of water in a porcelain dish and remove the finger.

The cleaning of the palladium standards is done by heating to redness in a porcelain dish for some minutes. That the weights stand this treatment well was found in an experiment in which the *RW* of a palladium weight was determined before and after 1 hour of heating. The results of double determinations were 2.17 and 2.17 μg before, and 2.17 and 2.17 μg after the treatment. This result does not give any information as to whether the standards are altered by daily wear and tear, but there is every reason to believe that they will be more constant than the polystyrene weights. An estimation of the effect of use in causing loss of RW of the latter was obtained by comparing our polystyrene standard with the standardized palladium weight. The polystyrene weight had been standardized by ZEUTHEN and constantly used for more than two years in the routine work in this laboratory. According to ZEUTHEN the RW of the standard should be 3.32 μg, while we found 2.91 μg, a loss of about 0.5 per cent per month. Although this means that the *RW* determinations have been wrong in absolute terms, it has no influence at all on the conclusions made from our experiments, since the results to be compared have always been made on the same day or with a few days interval.

The palladium weights were standardized on a specially constructed micro-balance made of Vycor glass, which will be described in the following section.

3.2. Standardization of palladium weights.

3.21. The Vycor balance.

In the construction of a micro-balance it was decided to use the suspension principle of PETTERSON (6). In this case, the beam has two short side arms to the ends of which are fused very fine quartz fibers attached to the balance frame. The construction of the balance is illustrated in fig. 1.

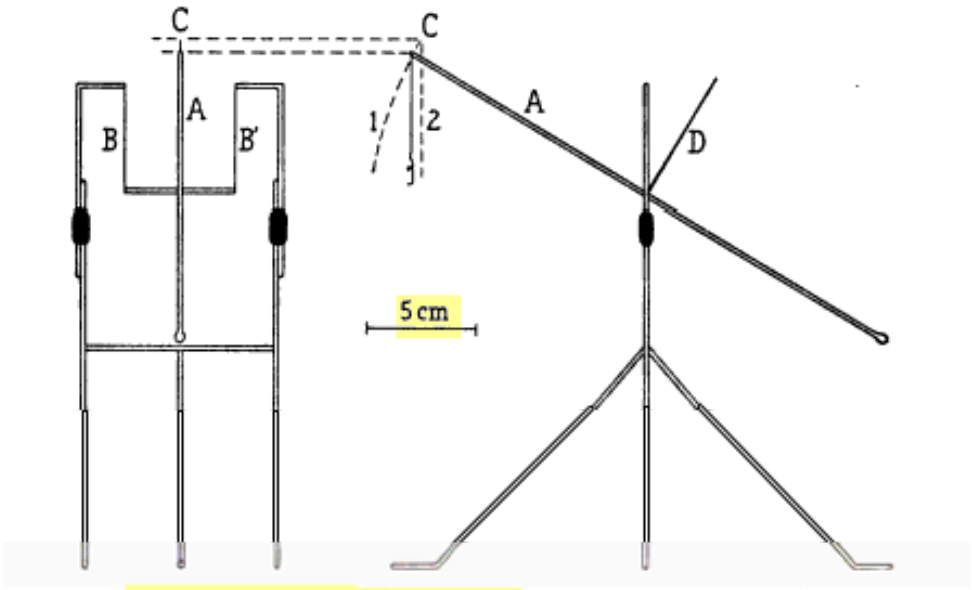

Fig. 1. The Vycor balance seen from front and from side.
A: beam. B and B′: quartz fibers. C: pointer. D: center of gravity regulator.
The pan suspension and pan are not drawn in the front view.
Lines 1 and 2: see text.

The frame was made of soft glass, and this was cemented by DE KHOTINSKY cement to Vycor glass tubes on which the beam was suspended. This way of attaching the beam to the stand made annealing impossible, which may be disadvantageous. The beam was made of Vycor glass tube about 1 mm in diameter. The beam and the pan were suspended by quartz fibers of about 30 μ thickness, which were made very thin at the first 1–2 mm from the point of attachment to the beam by gentle heating with an ordinary microgasflame. A Vycor glass rod tapering toward the free end was fused at the crosspoint of the side arms and the beam. This could be used to regulate the center of gravity by cutting off tiny pieces or by evaporating the glass by heating (see STEELE and GRANT (8)).

The pans were made of platinum foil (10 μ thick), to which was welded thin platinum wire, bent to form a hook. The weight of a pan was approximately 3 mg. The pan weights were adjusted by cutting off tiny pieces.

The balance was mounted in an old balance case , the arresting mechanism of which was transformed so as to be usable for the Vycor balance. The case was covered with insulating fiber plates, into which two holes were cut, one in the front side and one in the roof. The holes were covered with heat-absorbing glass. Inside the case was placed a piece of pitch-blende, and a dish with calcium chloride. The light source was a fluorescent lighting tube (7) placed in some distance from the roof window. The case was placed in a room, where the temperature was regulated to about $25.5^\circ \pm 0.25^\circ$. The readings were made with a cathetometer through the front window.

3.22. Weighings on the Vycor balance.

The period of the balance was about 20 seconds. 3-5 minutes after the balance had been set swinging, the resting position of the upper end of the pointer could be measured. If the readings were started immediately after the case had been opened it was noticed that the first values varied, while later ones approached an equilibrium value. Generally, therefore, the balance was left for half an hour after placing of the pan or standard, before the readings were started. The readings were continued –the balance being set swinging (by a movement of the arrest mechanism) after each reading– until at least two values of the equilibrium position were obtained which did not differ more than 0.01-0.02 mm (3 readings were generally enough). When the equilibrium position was determined, the standard and the loaded pan were exchanged (in the case of the palladium weights, the empty pan functioned as the standard) and another equilibrium position determined, and the difference between this and the former found. The determination of this difference was repeated until its value was established with a standard error of mean of less than 0.02 mm = ~0.01 μg. 3-5 weighings were sufficient.

The calibration of the balance was done by means of KCl-solutions pipetted onto the pan and evaporated. The pipetting was done by means of a constriction pipette with a volume of 2.23 μl, the standard deviation of delivery being 0.006 μl, or less than 3 per mille. This determination was made by iodometric titration of iodate solutions delivered by the pipette. The standard curve was a straight line, as is seen from fig. 2. 1 μg gives a displacement of 1.72 mm.

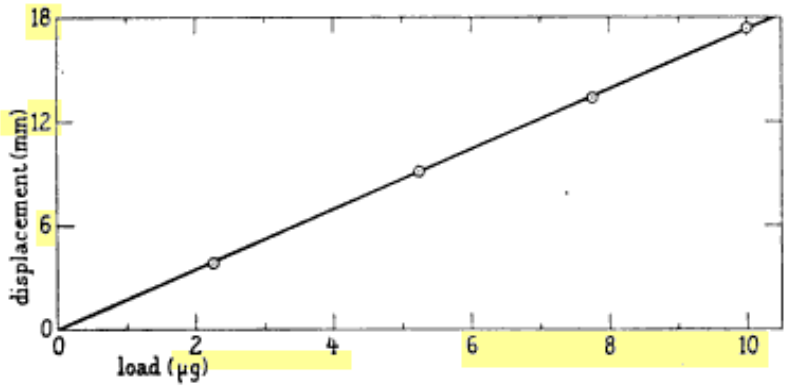

Fig. 2. Standard curve of the Vycor balance.

The form of the standard curve raises the question whether the Vycor balance is a displacement or a torsion balance. If it is a true displacement balance then the angle of displacement, and accordingly the arc described by the end of the beam (line 1 in fig. 1) are proportional to the load. But we were not measuring this arc, but its vertical projection (line 2). This would tend to make the apparent displacement for big loads relatively larger than for smaller ones, and accordingly the standard curve ought to be concave upwards. Now

it must be recalled that the readings were taken at the upper end of the pointer, which will tend to decrease the deviation from linearity. The simplest way to explain the complete linearity seems to be the assumption that the balance is essentially a displacement balance, but that torsion becomes increasingly effective with increasing loads. Unfortunately it was impossible to determine the shape of the standard curve with heavier loads than shown in fig. 2 since the range of the cathetometer used was only 20 mm.

To give an impression of the results obtained on the Vycor balance, the readings from weighing two weights have been summarized in table 1.

Table 1.

Weighings of the palladium weights on the Vycor balance.

Weight no.	1	2
Difference in equilibrium position due to the weight (mm)	7.67	6.96
	7.72	6.96
	7.74	6.97
	7.69	
Mean (mm)	7.705	6.963
Standard error of mean (mm)	0.016	0.003
Weight (μg)	4.45	4.02
Reduced weight (μg)	4.06	3.67

The reduced weights of the palladium standards are calculated from their weights by multiplication by $\frac{\varphi-1}{\varphi}$, where φ is the density of palladium. The value of the fraction is 0.913.

3.3. Weighing of the palladium standards on the diver balance.

The RW of the palladium weights was determined on the diver balance and the results obtained with the two different methods compared. These results are listed in table 2. The weight having *RW* of 4.06 μg has been used as standard in the diver balance weighings. The value 7.73 for weights 1+2 is obtained by addition. The weighings were done on three different days and with two different diver balances. The agreement between the two methods is satisfactory.

Table 2.

Comparison of the weighings on the Vycor balance and on the diver balance.

Weight no.	*RW* (μg) Vycor balance	*RW* (μg) diver balance		
1	4.06	(4.06)	(4.06)	(4.06)
2	3.67	3.66	3.68	3.66
1 + 2	(7.73)	7.70		

4. THE CHANGE OF THE EQUILIBRIUM PRESSURE OF THE DIVER BALANCE

4.1. The diver balance formula.

In his paper on the diver balance (9) ZEUTHEN uses the following formula for the calculation of *RW* from an observed change in equilibrium pressure:

$$(1) \qquad RW_x = RW_{st} \cdot \frac{1 - \frac{B}{B - p_x}}{1 - \frac{B}{B - p_{st}}},$$

where *x* and *st* refer to the object of unknown *RW* and the standard respectively, and *p* is the difference in equilibrium pressure between the loaded and empty balance. *B* is the barometric pressure and is conventionally put to 1000 cm BRODIE. A simple rearrangement of ZEUTHEN'S formula is

$$(2) \qquad RW_x = \left[\frac{RW_{st}(B - p_{st})}{p_{st}}\right] \cdot \frac{p_x}{B - p_x} = A \cdot \frac{p_x}{B - p_x},$$

where the average value of *A*, obtained from two independent weighings, may be used for calculation of unknown reduced weights.

An expression of similar form may be derived on basis of the three following equations:

$$(3) \qquad RW_x = W_x - v_x \cdot \varphi_M,$$

$$(4) \qquad \frac{W_D + W_x}{V_x + v_D + v_x} = \varphi_M, \quad \text{and,}$$

$$(5) \qquad V_x(p + \Delta p_x) = V_0 \cdot p.$$

Equation (3) is the definition of reduced weight, i. e. the RW of x is equal to the weight of x, W_x, minus the weight of the displaced medium, $v_x \cdot \varphi_M$, v_x, is the volume of x and φ_M the density of the medium (LINDERSTRØM-LANG and HOLTER (5)).

Equation (4) states that the "compound-density" of the diver balance and the load x must be equal to the density of the medium φ_M, when the diver is floating (LINDERSTROM-LANG (4), equation (49)). V_x, is the volume of the air bubble, W_D and v_D denotes the weight and volume of the balance.

In equation (5) is expressed that the product of pressure and volume of the enclosed air bubble is constant. V_0 is the volume of the air bubble when the balance is not loaded, and p the pressure, while Δp_x, is the change in pressure due to the load (Δp_x, is negative).

By combination of equations (3) and (4) we obtain:

$$RW_x = V_x \cdot \varphi_M - RW_D, \tag{6}$$

where RW_D is the reduced weight of the balance. If in this equation we put $RW_x = 0$, we will have for the unloaded diver balance:

$$RW_D = V_O \cdot \varphi_M = Z. \tag{7}$$

The reduced weight of the diver balance is called the "ZEUTHEN diver balance constant" and is denoted by Z. In our case, where φ_M is 1, the numerical value of Z (in μg) is seen to be equal to the volume of the air bubble (in μl $\times 10^{-3}$).

From (5), (6), and (7) we arrive at the following formula for the calculation of unknown reduced weights:

$$RW_x = \frac{p \cdot V_O \cdot \varphi_M}{p + \Delta p_x} - RW_D = RW_D \left(\frac{p}{p + \Delta p_x} - 1 \right) = Z \cdot \frac{-\Delta p_x}{p + \Delta p_x}. \tag{8}$$

The equation obtained (8) differs from the one employed by one employed by ZEUTHEN, transformed as in (2) in one respect only; viz. B (the barometric pressure) in (2) has been replaced by p, the pressure of the air bubble of the empty floating diver.

Before the expression p is discussed, it may be worth mentioning, that the fact that ZEUTHEN used 1000 instead of p, does not mean that the values of RW he calculated were wrong. This is easily seen from the transformed equation (2), where a replacing

of B with p, even if these differ as much as 5 per cent, cannot change the ratio $\frac{B-p_{st}}{B-p_x}$ noticeably.

As to the actual determination of p, it must be recalled that it consists of 4 terms, namely the constant pressure in the air bottle, the difference between this pressure and the one in the diver vessel when the diver is floating (the actual reading), the pressure of the water from the surface of the floating medium to the height of the air bubble, and the excess pressure due to surface forces acting on the air bubble surface. The first term may be kept constant simply by not equalizing to the atmosphere. The third term has a constant value of about 2 cm H_2O. The value of the last term will change with the radius of the air bubble, but the variation is negligible. The numerical value of this pressure has been calculated by ZEUTHEN to be ~5 cm H_2O. The second term is the pressure difference indicated by the manometer, when the empty balance is floating. This term, denoted p_D, is generally negative, and the only one which cannot be kept constant (see section 4.2). When the value of p_D changes, then p, which is put equal to $1000 + p_D$, will change by the same amount.

The value of Z is obtained in the following way: The equilibrium pressure of the empty balance, p_D, is measured. The diver balance is then loaded with a standard weight, and the new pressure difference between the air bottle and the diver vessel determined. This pressure difference is equal to $p_D + \Delta p_{st}$. The value of Δp_{st} is found by substraction. Generally p_D is measured again, when the standard has been taken off, and the value of p_D at the time when the loaded balance was measured, is interpolated. Z may now be computed from (8):

$$Z = RW_{st} \cdot \frac{p + \Delta p_{st}}{-\Delta p_{st}} = RW_{st} \cdot \frac{1000 + p_D + \Delta p_{st}}{-\Delta p_{st}}. \tag{9}$$

From equation (8) it may be seen directly that the sensitivity of a balance is a function of the constant Z, the smaller Z is, the larger Δp will be for the same RW. This is one advantage of employing the diver balance constant, that one may directly compare the sensitivity of different balances standardized with different weights. Another advantage is that it enables one to detect changes in the RW of the standard or the balance. If the value of the constant, Z, determined before a series of weighings, differs more than ± 0.5 per cent from previous determinations, then there is reason to clean either standard or balance, or both.

If $\mathcal{Z}$ is found to have increased it will mean that the *RW* of the balance has increased or the *RW* of the standard has decreased, while a smaller value of $\mathcal{Z}$ will indicate the reverse possibilities with respect to changes in *RW*.

If on the other hand $\mathcal{Z}$ has been found to agree with previous values there is no reason to weigh the standard again when the weighings of the unknowns are finished, as proposed by ZEUTHEN (9).

4.2. TCauses of the change in equilibrium pressure p_D.

ZEUTHEN (9) has discussed the reasons why the empty diver balances gets gradually "heavier", that is, the value of p_D, decreases, and reaches the conclusion that the phenomenon probably is due to a combined action of the factors, 1) a slow loss of air by diffusion from the air bubble and 2) a slow increase of the reduced weight of the balance due to accumulation of dust particles etc. on the surface of the diver balance.

This problem may be attacked by determining Z from day to day, while the balance is getting "heavier". If we can assume that the standard does not change its RW, then Z will remain constant if the change in p_D is due to air loss, while it will increase if the RW of the balance increases.

A comparison of equations (2) and (9) shows that the value of A must increase gradually from one refilling of the diver balance to the next, even if the RW of the balance remains constant, because the decrease of p_D is not included in (2). The value of A will of course return to the initial level, when the air loss has been replaced by refilling the balance, if the RW of the diver balance is constant.

The results obtained by determining Z and A of a balance at various times during $1\frac{1}{2}$ month are shown in table 3. It is seen that Z is oscillating around an average value. The variation in Z corresponds to 0.8 per cent, which corresponds to ± 1 mm BRODIE

Table 3.

Comparison of the constancy of Z and A. The diver has been refilled 1–6, 13–6, 20–6, and 11–7.

Date	1–6	2–6	3–6	13–6	14–6	15–6	16–6
Z (μg)	108.7	108.1	108.0	108.0	108.8	108.0	108.2
A	110.9	111.2	111.8	109.5	109.5	110.9	112.1

Date	20–6	11–7	12–7	13–7	14–7	15–7
Z (μg)	108.5	107.7	107.5	107.9	107.7	107.2
A	110.4	109.8	110.0	110.9	111.2	111.5

in Δp_{st}. The greatest variation in A is from 109.5 to 112.1, that is 2.5 per cent, only three times the variation in Z, but the values are not scattered arbitrarily, but changing in the expected direction. The maximum change in A corresponds to a change in p_D of only 27 cm BRODIE, but changes in p_D of 50 cm are not unusual before refilling is necessary, so that greater changes in A may occur. The found constancy of Z (and the return of A to the initial level after refilling) seems to allow the conclusion that the "gradual increase of RW" is only apparent and due to loss of air.

The average value of Z in the last period is slightly lower than in the first periods, indicating some loss of material (sliver of glass) from the balance during the fourth refilling procedure.

A change in temperature of the air enclosed in the diver balance will also change p_D. This effect amounts to 1000/293 = ~3.5 cm BRODIE per degree, thus changing the value of A by ~0.4 per cent while Z, the RW of the balance, remains unchanged.

> A continuous gain in weight may have a cause not yet mentioned: Growth of micro-organisms. During our first work with the diver balance it very often happened that the balance got too heavy in 2–3 days, and as a rule it was possible to demonstrate microscopically growth of micro-organisms, usually moulds, on the balance. Since then we have tried to work "semi-sterile", the PRINGSHEIM Solution was filtered germ-free, the amoebae were never taken directly from the cultures (Andresen (1)), but washed by transfer to PRINGSHEIM Solutions. From time to time the pipettes used for transfer of amoebae and standard were gently flamed. In this way it has been possible to eliminate this source of inconstancy, which, when occurring, of course will influence the value of Z.

The author wishes to express his sincerest thanks to Dr. H. HOLTER for his interest and encouragement, his suggestions and criticism, all of which have contributed very much to this work, and to professor K. LINDERSTRØM-LANG and Dr. E. ZEUTHEN for a critical revision of the manuscript. My best thanks are also due to Miss GRETHE NØRBY, who with great patience has made a large number of diver balances, and assisted in many of the experiments.

SUMMARY

A slight modification in making the ZEUTHEN diver balance is described.

Weights made of very fine palladium wire have been introduced as standards for weighing on the diver balance. These were

weighed on a specially constructed micro-balance, having a sensitivity of 0.01 μg. The building of this balance is briefly outlined.

A slightly different way of calculating the results obtained on the diver balance has been introduced. This method employs a constant, the "ZEUTHEN diver balance constant", Z, the numerical value of which is equal to the reduced weight in μg of the diver balance.

The constancy of Z during an interval, where the diver balance gets gradually "heavier", has led to the conclusion that the main cause of the change of equilibrium pressure of the diver balance is air loss.

REFERENCES

1. ANDRESEN, N.: Cytoplasmic components in the amoeba *Chaos chaos* Linné. Compt. rend. Lab. Carlsberg, Sér. chim. **24**, 139–184 (1942).
2. HOLTER, H. and S. LØVTRUP: Proteolytic enzymes in *Chaos chaos*. Compt. rend. Lab. Carlsberg, Sér. chim. **27**, 27–62 (1949).
3. LEVI, H. and E. ZEUTHEN: Micro weighing in the gradient tube. Compt. rend. Lab. Carlsberg, Sér. chim. **25**, 273–287 (1946).
4. LINDERSTRØM-LANG, K.: On the theory of the Cartesian diver micro respirometer. Compt. rend. Lab. Carlsberg, Sér. chim. **24**, 333–398 (1943).
5. LINDERSTRØM-LANG, K. and H. HOLTER: Die enzymatische Histochemie. In E. Bamann and K. Myrbäck: Die Methoden der Fermentforschung, pp. 1132–1162. Leipzig (1940).
6. PETTERSON, H.: A new microbalance and its use. (Diss.) Göteborg (1914).
7. RICHTER, K. and B. BARFOD: A new „daylight"-device for titrations. Acta Chem. Scand. **2**, 291–292 (1948).
8. STEELE, B. D. and K. GRANT: Sensitive microbalances and a new method of weighing minute quantities. Proc. Roy. Soc. London. A, **82**, 580–594 (1909).
9. ZEUTHEN, E.: A Cartesian diver balance weighing reduced weight (*R.W.*) with an accuracy of 0.01 ± *y*. Compt. rend. Lab. Carlsberg, Sér. chim. **26**, 243–266 (1948).

Imprimerie de Bianco Lunos Bogtrykkeri, Copenhague (11 1950)